Effects of foliar spraying of silicon and phosphorus on rice (*Oryza sativa*) plants and their resistance to the white-backed planthopper, *Sogatella furcifera* (Hemiptera: Delphacidae)

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Abstract: 【Aim】 Silica strengthens disease resistance and improves environmental stress tolerance in plants. We examined the changes in the silicon content and some biochemical substances in rice leaves following foliar spraying of silicon and phosphorus, and the effects of spraying silicon on a population of the white-backed planthopper (WBPH), *Sogatella furcifera*, in order to further understand the enhancement of innate rice resistance to pests by exogenous elements. 【Methods】 After foliar spraying of silicon, phosphorus, and silicon + phosphorus at the tillering stage of rice, the contents of silicon, oxalic acid, and soluble sugar in rice leaves were measured. After foliar spraying of silicon, the silicon cells around the stomata of rice leaf were observed under transmission electron microscopy (TEM), and the population growth parameters of WBPH fed on treated rice were also examined. 【Results】 The silicon content in rice leaves in foliar spray treatment with 20 or 40 mg/L silicon or silicon + phosphorus increased significantly compared to that in the control ($P < 0.05$). The silicon contents on both the upper and lower sides of rice leaves in foliar spray treatment with 40 mg/L silicon + 40 mg/L phosphorus increased by 116% and 104.4%, respectively, compared to that in the control ($P < 0.01$). There were more silicon cells around the stomata of treated rice leaves. The oxalic acid content in rice leaves at 3 and 6 d after foliar spray treatment with silicon + phosphorus increased significantly. The soluble sugar content in rice leaves for most silicon + phosphorus combinations increased. The number of eggs laid by per female of *S. furcifera* fed on rice leaves sprayed with 40 mg/L silicon decreased significantly ($P < 0.05$). 【Conclusion】 The foliar spraying of silicon + phosphorus enhances the innate resistance of rice to pests and induces an increase of resistant substances in rice plants, and decreases the number of eggs laid by the WBPH females.

Key words: *Sogatella furcifera*; foliar spray; silicon; phosphorus; rice leaf; silicon content; oxalic acid content; fecundity

1 INTRODUCTION

The occurrence of rice pests has been a serious threat to food safety in China. Rice insect pests have resulted in a 15% - 25% loss of rice yield if effective controls are not implemented (Zhu and Cheng, 2013). The area of rice hopperburn reached 6 600 ha in Jiangsu Province alone in 2005 (Gao *et al.*, 2006), and parts of rice paddy fields completely lost their yield. Therefore, rice insect pest control is a key management tactic for ensuring food safety. Insecticides are still an effective control practice; however, rice planthoppers frequently become resistant to insecticides. For example, the resistance of the brown planthopper (BPH) *Nilaparvata lugens* Stål to buprofezin increased significantly and reached a moderate resistance level in the main rice-growing regions of China (11.3 to 23.4-fold) in 2010, and 80% of the populations reached a high resistance level (40.7 to 119.7-fold) in 2011. All monitored populations remained moderately to extremely resistant to imidacloprid (82.3 to 1 935.8-fold) (Wang *et al.*, 2013). The Wuxi (Jiangsu) and Huzhou (Zhejiang) populations of the small brown planthopper (SBPH) *Laodelphax striatellus* Fallén developed a high level of resistance to imidacloprid (79.6 and 44.6-fold, respectively) (Ma *et al.*, 2007). For the white-backed planthopper (WBPH), *Sogatella furcifera* (Horváth), most populations developed moderate resistance to buprofezin in eastern China (up to 25-fold). Approximately 32% of the field populations exhibited moderate resistance to imidacloprid (Su *et*

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al., 2013). In addition, sub-lethal doses of several pesticides (*e. g.*, triazophos, imidacloprid and buprofezin) not only stimulated the fecundity of BPH (Azzam *et al.*, 2009), but also enhanced its thermotolerance (Ge *et al.*, 2013) and flight capacity (Zhao KF *et al.*, 2011). Thus, rice pest control that relies only on pesticides is not sustainable. We consider the enhancement of the innate resistance of rice plants to pests regulated by exogenous factors as a more sustainable approach for rice pest management.

Silicon, the second most abundant element in the Earth's crust following oxygen, is ubiquitous in soil and constitutes 50% – 70% of the total soil dry weight in the form of silica, SiO_2 (Isa *et al.*, 2010). In rice, silica not only significantly strengthens disease resistance (de Camargo *et al.*, 2013), but also enhances growth and yield (Isa *et al.*, 2010; Kim *et al.*, 2012; Zhao *et al.*, 2013) and alleviates growth inhibition induced by heavy metals (Nwugo and Huerta, 2008). It also improves environmental stress tolerance in plants (Ma, 2004). Based on these properties, silicon can ecologically regulate the incidence of rice pest occurrences. However, the mechanisms of changes in biochemical substances in rice plants following silicon foliar sprays and the effects on pests need to be clarified upon further study. The WBPH is a serious rice pest in Asian countries. It causes damage to rice production by directly sucking the sap and transmitting pathogenic viruses, *e. g.* southern rice black-streaked dwarf virus, SRBSDV (Zhang *et al.*, 2008; Zhou *et al.*, 2008) and has also developed resistance to insecticides, *e. g.*, buprofezin, thus its management is difficult (Su *et al.*, 2013). In addition, it has been demonstrated that the silicon content in rice plants is closely related to the resistance of rice to pests (Wang *et al.*, 2008). Therefore, to develop an approach for the ecological regulation of rice pests by the enhancement of the innate resistance of rice plants, we conducted experiments of foliar spray of silicon fertilizer or the combination of silicon with phosphorus to examine the changes in the silicon content in leaves and some biochemical substances, as well as the effects on the WBPH.

2 MATERIALS AND METHODS

2.1 Rice variety, insects and silicon and phosphorus fertilizers

The rice (*Oryza sativa* L.) variety Huaidao 9 (japonica rice) was used in the trials. This variety of rice was selected because it is commonly planted in Jiangsu Province, China. Seeds were sown outdoors in standard rice-growing soil in cement tanks (length × width × height = 200 cm × 100 cm × 60 cm). When the seedlings reached the 6-leaf stage, they were transplanted into plastic pots (32 cm in diameter and 28 cm in height), with 2 hills per pot and four plants per hill. The rice plants used in the experiments were at the tillering stage.

2.2 Insect stock

A laboratory strain of *S. furcifera*, originally obtained from natural populations in the Yangzhou University farm (Yangzhou, Jiangsu, China) (32° 24′7.48″N, 119° 22′59.32″E), was reared using the susceptible variety Shenyou 1 for five generations at 26 ± 1℃, with 70% – 80% humidity and a 16L : 8D photoperiod in a greenhouse at Yangzhou University.

2.3 Silicon and phosphorus fertilizers

Silicon (50% SiO_2) and phosphorus (P_2O_5) fertilizers were provided by Bio Huma Netics, Inc. (Phoenix City, Arizona, USA) and Tianjin Junjia Laboratory Reagent Co. Ltd. (Tianjin, China), respectively.

2.4 Experimental design

To examine the combined effects of silicon and phosphorus, foliar spraying of silicon and a mixture of silicon and phosphorus were applied at the tillering stage using a Jacto sprayer (Maquinas Agricolas Jacto S. A., Brazil) equipped with a cone nozzle (1-mm diameter orifice, pressure of 45 psi, flow rate of 300 mL/min). The level of each element and its combinations are shown in Table 1. Each treatment and the control (water foliar sparying) was replicated four times.

Table 1 Combinations of foliar spraying of silicon and phosphorus

Silicon (S) (mg/L)	Phosphorus (P) (mg/L)	Combination
0 (S_1)	0 (P_1)	S_1P_1
	20 (P_2)	S_1P_2
	40 (P_3)	S_1P_3
20 (S_2)	P_1	S_2P_1
	P_2	S_2P_2
	P_3	S_2P_3
40 (S_3)	P_1	S_3P_1
	P_2	S_3P_2
	P_3	S_3P_3

2.5 Transmission electron microscopy (TEM) observation of silicon cells of rice leaves or sheaths

The 4th leaf from the top of rice plants at 6 d after treatment was cut for each treatment and control plant. The middle of the leaf (approximately 1 cm^2) was soaked in 4% glutaraldehyde, washed thrice with 0.2 mol/L phosphate buffer solution (pH 7.0)

for 3 min each time, dehydrated with graded concentrations of ethanol (30%, 50%, 70%, 80%, 90%, and 100%), sprayed with gold plating using a vacuum ion sputter after drying with CO_2, and then observed and photographed with a Tecnai 12 TEM (Philips-FEI Co. Ltd., Holland). The relative content of silicon was calculated by X-ray spectroscopy.

2.6 Measurement of soluble sugar and oxalic acid contents in rice leaves

To assess the biochemical changes in the treated rice plants, the contents of soluble sugar and oxalic acid in four leaves were measured at 3 and 6 d after foliar spraying. Soluble sugar levels were measured using the method of Zhang *et al*. (2004). One gram of leaves was weighed and ground, placed in a 20 mL test tube, extracted in boiling water for 10 min after the addition of 10 mL of distilled water, cooled, and filtered. The supernatant was put in a 100-mL measuring flask and filled up to 100 mL with distilled water. One milliliter of extraction solution was absorbed, placed in a test tube, heated in boiling water for 10 min after the addition of 5 mL anthrone, and cooled. The absorbance at 620 nm was detected with the UV755B spectrometer (Shanghai Precision Science Instrument Ltd. Co.). A standard curve was established with glucose.

The trichloride titanium development method was used to measure oxalic acid content (Zhang *et al*., 1997). One gram of leaves was weighed, ground, washed with 10 mL ultrapure water, and placed in a 50-mL flask. Active carbon was added to the supernatant for decolorization, and the carbon was then separated from the solution by centrifugation. The decolorization step was repeated using the above method until the solution reached a colorless or milk-white state; 0.15 mL of trichloride titanium was then added to 3 mL of the decolorized solution and centrifuged. The absorbance was measured at 400 nm using the UV755B spectrometer. A standard curve was established using 99.5% oxalic acid (Shanghai No. 4 Reagent Co. Ltd., Shanghai, China).

2.7 Effects of silicon foliar sprays on the WBPH

Forty parts per million of silicon was sprayed on the potted rice at the tillering stage, and no spray was used as a control. Ten centimeter-long rice stems at 3 d after spraying were cut and placed into a glass cup (10 cm in height, and 5 cm in diameter); 30 1st instar nymphs were then released. Insect mortalities were checked at 2 h after the release of the nymphs, and dead nymphs (if any) were replaced with live nymphs at the same age to maintain a given density. The cups were placed in a bioculture box at 27 ± 1℃, RH 75%, and photoperiod of 16L: 8D. The development of the nymphs was recorded until adult emergence. After emergence, one pair of female and male adults was placed and mated in a glass cup containing treated or untreated rice stems for oviposition. The rice stems were changed every 2 days, and the number of eggs laid was counted under a microscope. Eggs were scraped from the leaf sheaths and blades using a pin. Each treatment and the control were replicated 10 times.

2.8 Statistical analysis

Normal distributions and homogeneity of variance (determined using the Bartlett test) were verified before performing analysis of variance (ANOVA) tests. A two-way ANOVA (silicon concentration and phosphorus concentration) was performed for changes in biochemical substances or silicon content after the foliar spray. A one-way ANOVA for the effect of the silicon foliar spray on the WBPH was also performed. Multiple comparisons of means were conducted based on Fisher's protected least significant difference (PLSD) test at $P<0.05$. All the analyses were conducted using the data processing system (DPS) of Tang and Feng (2002).

3 RESULTS AND ANALYSES

3.1 Changes in the silicon content in rice leaves after foliar spray of silicon and phosphorus

Data from Table 2 showed that foliar spraying of different concentrations of silicon and phosphorus significantly influenced the silicon content on the upper and lower sides of leaves ($F=206.3$, $df=2, 26$, $P=0.0001$ for silicon concentration; $F=28.8$, $df=2, 26$, $P=0.0001$ for phosphorus concentration) (Table 3), and the two variables interacted significantly ($F=27.6$, $df=4, 26$, $P=0.0001$). The silicon content increased with the increase of silicon and phosphorus concentrations. Multiple comparisons showed that the silicon content on the upper and lower sides of leaves in foliar spray treatment with 40 mg/L silicon + 40 mg/L phosphorus were significantly higher than that of the control and other concentrations (Table 2). In addition, the silicon content in leaves sprayed with silicon + phosphorus were significantly higher than that of the control.

In addition, TEM observation showed that leaves treated with the silicon foliar spraying have more silicon cells and higher silicon cell density around their stomata (Fig. 1).

Table 2 Relative content (%) of silicon on the upper and lower sides of rice leaves after foliar spray of silicon and phosphorus

Treatment combination	Leaf upper side	Leaf lower side
S_1P_1	5.43 ± 0.19 f	6.11 ± 0.15 d
S_1P_2	8.10 ± 0.31 de	9.09 ± 0.42 bc
S_1P_3	7.16 ± 0.09 e	8.41 ± 0.29 c
S_2P_1	8.90 ± 0.18 cd	9.68 ± 0.41 bc
S_2P_2	10.00 ± 0.06 b	10.62 ± 0.15 b
S_2P_3	9.04 ± 0.11 bcd	9.87 ± 0.48 bc
S_3P_1	9.95 ± 0.11 bc	10.36 ± 0.24 b
S_3P_2	9.30 ± 0.19 bc	12.38 ± 0.09 a
S_3P_3	11.74 ± 0.41 a	12.49 ± 0.59 a

The means ± *SD* followed by different letters within the same column are significantly different based on Fisher's protected least significant difference (PLSD) test at $P < 0.05$.

3.2 Changes in the contents of oxalic acid and soluble sugar in rice leaves after foliar spraying of silicon and phosphorus

Figs. 2 (A, B) showed that the silicon and phosphorus concentrations and their interaction effects (except for day 6 for S × P) significantly influenced the oxalic acid content (Table 3). Multiple comparisons showed that most combination treatments significantly increased the oxalic acid content at 3 and 6 d after foliar spraying (3 and 6 DAS) compared to the control (S_1P_1) (Fig. 2: A, B), especially S_2P_2. In addition, Silicon levels were closely related to the oxalic acid content. For example, the combinations of phosphorus-only spray (S_1P_2 and S_1P_3) at 3 and 6 DAS did not significantly increase the oxalic acid content, indicating that silicon plays a key role in the increase in oxalic acid.

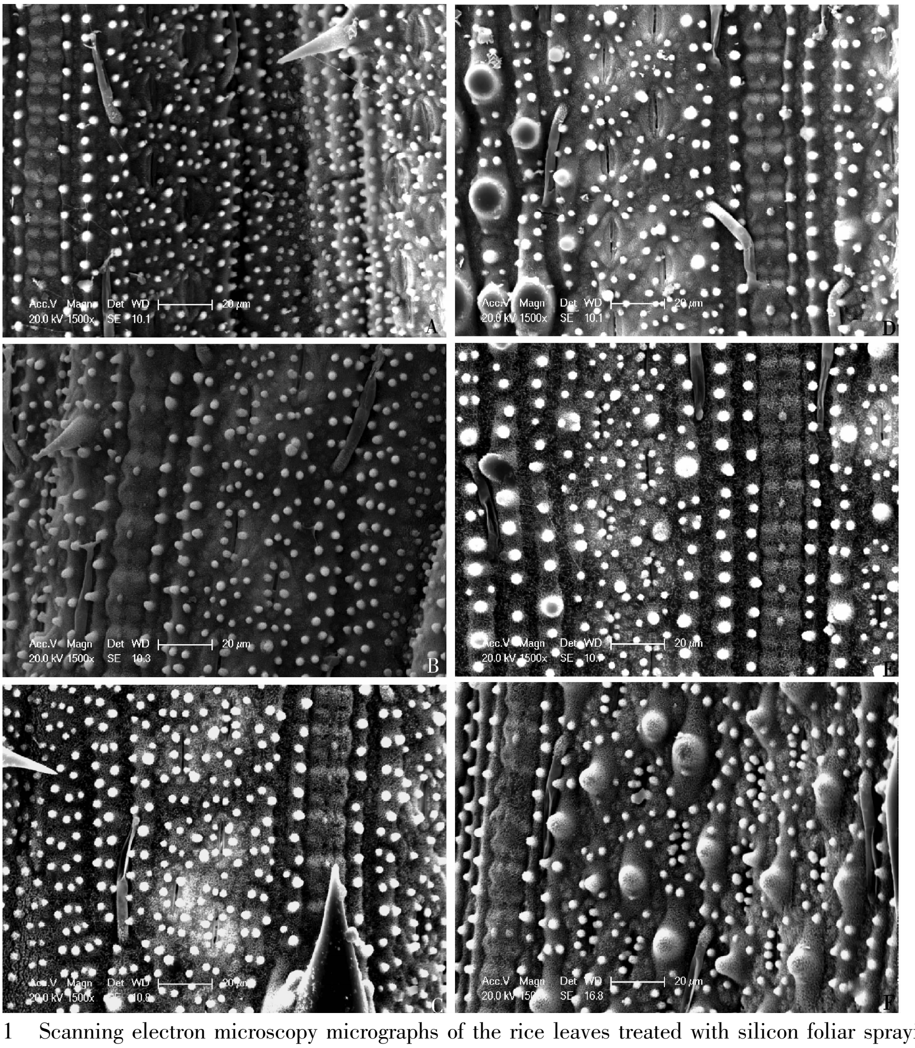

Fig. 1 Scanning electron microscopy micrographs of the rice leaves treated with silicon foliar spraying

A: Leaf upper side of the control; B: Leaf upper side in foliar spray treatment with 20 mg/L silicon; C: Leaf upper side in foliar spray treatment with 40 mg/L silicon; D: Leaf lower side of the control; E: Leaf lower side in foliar spray treatment with 20 mg/L silicon; F: Leaf lower side in foliar spray treatment with 40 mg/L silicon.

Table 3　ANOVA of contents of silicon, oxalic acid, and soluble sugar in rice leaves following foliar spraying of silicon (S) + phosphorus (P)

Item studied	Days after spraying	Variance source	*df*	*F*-value	*P*-value
Content of silicon on upper leaf	6	S(A)	2	206.3	0.0001
		P(B)	2	28.8	0.0001
		A × B	4	27.6	0.0001
Content of silicon on lower leaf	6	A	2	55.6	0.0001
		B	2	2.64	0.0986
		A × B	4	9.03	0.0003
Content of oxalic acid	3	A	2	6.7	0.0041
		B	2	13.2	0.0001
		A × B	4	2.7	0.0494
	6	A	2	16.9	0.0001
		B	2	5.9	0.0074
		A × B	4	2.0	0.1129
Content of soluble sugar	3	A	2	8.9	0.0011
		B	2	3.3	0.0537
		A × B	4	3.9	0.0128
	6	A	2	14.3	0.0001
		B	2	6.8	0.0039
		A × B	4	6.5	0.0008

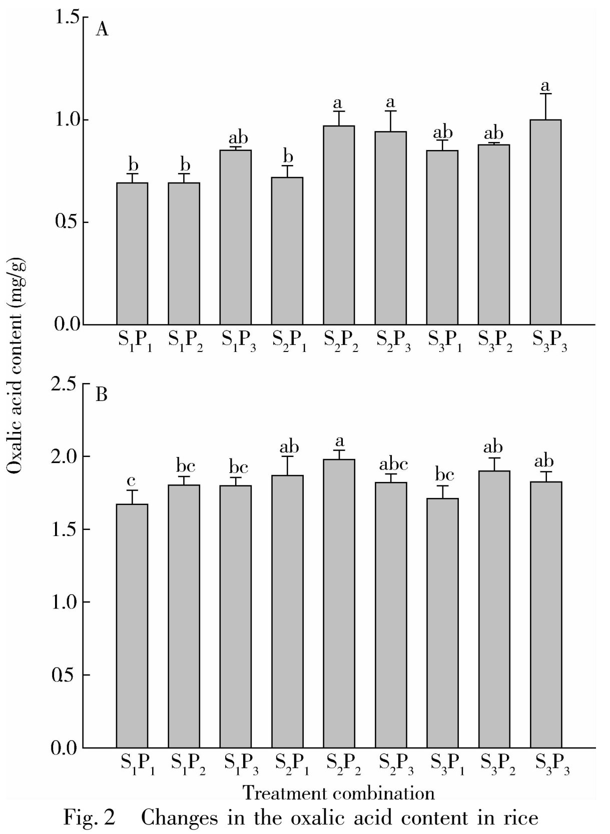

Fig. 2　Changes in the oxalic acid content in rice leaves at 3 d (A) and 6 d (B) following foliar spraying of silicon (S) + phosphorus (P)

Data in the figure are represented as mean ± *SE*. Different letters above bars represent significant differences at the 5% level by Fisher's protected least significant difference (PLSD) test. DAS is days after foliar spray. S_1, S_2, and S_3 are 0, 20, and 40 mg/L silicon, and P_1, P_2, and P_3 are 0, 20, and 40 mg/L phosphorus, respectively. The same for the following figures.

Fig. 3 (A, B) indicated that the silicon and phosphorus concentrations and their interaction effects (except for 6 DAS) significantly influenced the soluble sugar content (Table 3). Multiple comparisons showed that only S_2P_2, S_3P_1, and S_3P_3 significantly increased the soluble sugar content at 3 DAS compared to the control (S_1P_1) and other combinations (Fig. 3: A, B). Most spray combinations at 6 DAS significantly increased the soluble sugar content compared to the control.

3.3　Effects of silicon on the WBPH

The foliar spraying of silicon did not influence the developmental duration (DD) and survival rate (SR) of the WBPH ($F = 1.1$, $df = 1, 19$, $P = 0.31$ for DD; $F = 2.2$, $df = 1, 19$, $P = 0.16$ for SR) (Figs. 4, 5). However, the foliar spraying of silicon significantly decreased the number of eggs laid ($F = 10.5$, $df = 1, 38$, $P = 0.002$) (Fig. 6) by 30.5%, indicating that the foliar spray of silicon is adverse to the fecundity of the WBPH.

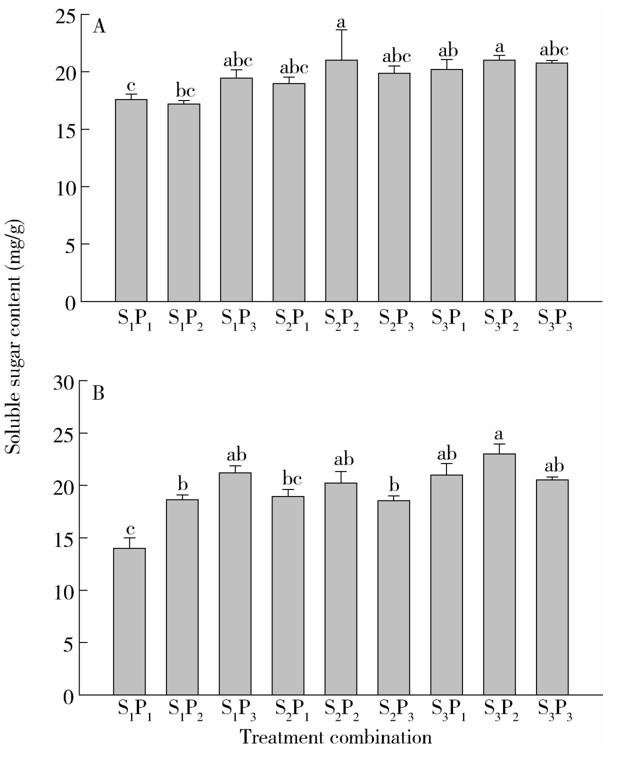

Fig. 3　Changes in the soluble sugar content in rice leaves at 3 d (A) and 6 d (B) following foliar spraying of silicon (S) + phosphorus (P)

4　DISCUSSION

Rice is the core of the paddy ecological system. There are very closely interplayed relationships between rice plants and pests. The population growth of pests is significantly influenced by the status of

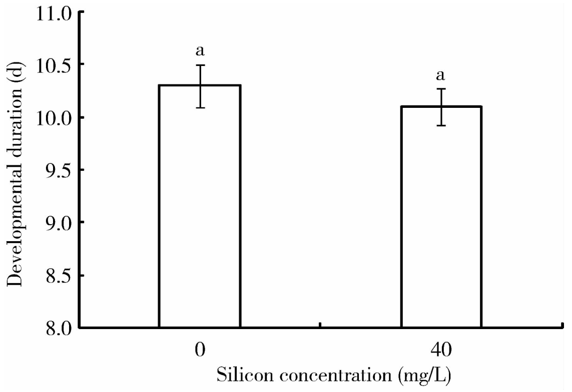

Fig. 4 Developmental duration of *Sogatella furcifera* following foliar spraying of 40 mg/L silicon

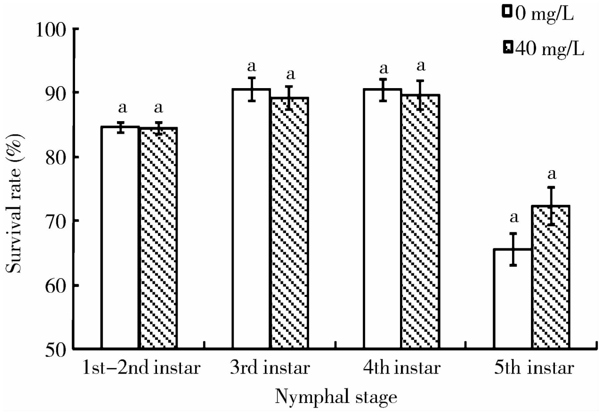

Fig. 5 Survival rate of *Sogatella furcifera* following foliar spraying of 40 mg/L silicon

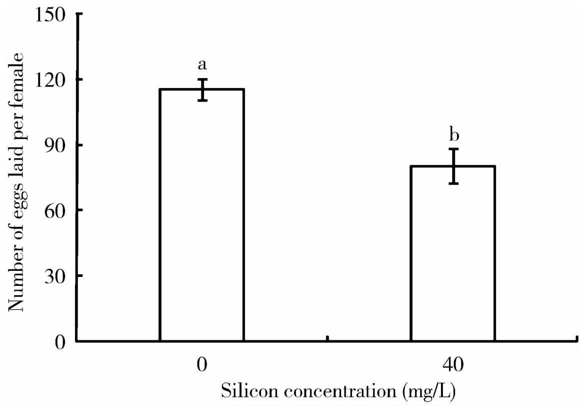

Fig. 6 Number of eggs laid by *Sogatella furcifera* female adult following foliar spraying of 40 mg/L silicon

rice plants, including their resistance and nutritional levels. Therefore, the regulation of the enhancement of the innate rice resistance to pests is a key technique of the ecological management of pests (Liu *et al.*, 2003; Voleti *et al.*, 2008). The present findings showed that foliar sprays of silicon or silicon + phosphorus significantly increased contents of both silicon and oxalic acid in rice leaves, and decreased the number of eggs laid by the WBPH females. Silicon has various beneficial physiological functions in rice plants. For example, silicon taken up by rice plants and deposited in plant epidermal cells formed a bilayer structure of silicon-cuticle, inhibited transpiration, and enhanced the photosynthetic rate (Xin and Zhang, 1998). Silicon fertilization increased the vigor of rice roots and enhanced the uptake of nutrients by roots (Zhang *et al.*, 2004), which is the basis of the enhancement of rice resistance to pests. Many studies have demonstrated that the silicon content in leaves is positively related to the resistance of plants to pests. For example, silicon was deposited on epidermal cells, formed siliceous cells, and as a physical barrier inhibited the infection of spores of fungi (Kim *et al.*, 2002). The application of silicon fertilizer inhibited stem borer damage by the sugarcane borer *Eldana saccharina* Walker and reduced the larval weight (Keeping and Meyer, 2002). Wang *et al.* (2008) reported that the silicon content in rice leaves is closely related to the resistance of rice to the rice leaffolder *Cnaphalocrocis medinalis* (Guenée). The physical mechanisms of the effect of silicon on insect pests involve in increasing wearing of the insect maxilla and in reducing digestive capacity of the insects (Hunt *et al.*, 2008). The present experiment showed that foliar sprays containing silicon significantly decreased the number of eggs laid by the WBPH, which may be related to significant increases in the silicon and oxalic acid contents in rice leaves following foliar sprays. Oxalic acid is the most active anti-feedant administered as a free acid or salt (Nagata and Hayakawa, 1998) and is considered to be relevant to rice resistance to planthoppers (Yoshihara *et al.*, 1980). In addition, foliar sprays of silicon or silicon + phosphorus significantly increased the soluble sugar content in rice plants in the present study. The relationship of soluble sugar and rice resistance to planthoppers varies with planthopper species. Yu *et al.* (1989) reported that a higher soluble sugar content in rice plants is unsuitable for the WBPH. However, the resistance of rice to the small brown planthopper *L. striatellus* was reduced with an increase in soluble sugar content (Liu *et al.*, 2007).

Phosphorus is also used as a foliar spray fertilizer. Tomato plants sprayed with Nutri-Vant-PeaK [95% monopotassium phosphate (MKP) and 5% Ferti-Vank] were taller and the yield was significantly higher than the control plants, indicating that the application of foliar phosphorus

nutrient via Nutri-Vank-PeaK is beneficial for greenhouse tomato production (Chapagain and Wiesman, 2004). The application of MKP efficiently suppressed powdery mildew, as expressed by the inhibition of development of new sporulating colonies, as well as the conidial production of the fungus on infected tissue (Reuveni *et al*., 1998). At the heading stage, 71.1% phosphorus (KH_2PO_4) of the foliar spray was absorbed by the leaf blades of rice. Phosphorus foliar spray increased the photosynthetic rate, root activity, grain-filling rate, and grain yield. In addition, phosphorus foliar spray is beneficial for the yield and quality of winter wheat (Zhao GC *et al*., 2011). The present findings also revealed significant interactions between the silicon and oxalic acid contents following phosphorus and silicon sprays. For example, the leaves treated with 40 mg/L silicon + 40 mg/L phosphorus had the highest silicon content (Table 1), while those treated with 20 mg/L silicon + 20 mg/L phosphorus had the maximum oxalic acid content [Figs. 1, 2 (A)], indicating that foliar spraying of 20 – 40 mg/L silicon + 20 – 40 mg/L phosphorus was the optimum combination for enhancing the innate resistance of rice to pests. The effects of the silicon and phosphorus foliar spraying on other pests in addition to WBPH need to be further investigated.

In summary, regulation of the enhancement of the innate resistance of rice to pests by exogenous factors (*e. g.*, silicon or phosphorus) is an innovative feature of pest sustainable management (PSM) because the enhancement of the resistance of rice plants is a core of PSM.

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叶面喷施硅和磷对水稻及其抗白背飞虱的影响

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摘要:【目的】硅可以增强植物的抗病性和对环境胁迫的耐受性,本实验检测了水稻叶面喷施硅和磷后叶片中硅和两种次生物质含量的变化以及喷硅对白背飞虱 *Sogatella furcifera* 种群的影响,旨在阐明外源元素施用是否会提高水稻的抗虫性。【方法】采用对分蘖期水稻进行硅肥、磷肥、和两者混合的喷施处理,测定比较了水稻叶片正面和反面硅含量、草酸含量和可溶性糖含量,同时检测了喷施硅肥后水稻叶片硅化细胞数量和取食处理水稻后白背飞虱种群增长的参数。【结果】20 和 40 mg/L 硅或硅 + 磷混合施用后,水稻叶片中的硅含量比对照显著增加($P<0.05$)。在 40 mg/L 硅 + 40 mg/L 磷喷施处理后,水稻叶片正反面的硅含量分别比对照增加了 116% 和 104.4%。扫描电镜结果显示,处理后的水稻叶片上气孔周围硅化细胞明显增加。此外,硅和磷喷施后 3 d 和 6 d,水稻叶片草酸含量显著增加($P<0.01$)。40 mg/L 硅处理后的水稻上饲养的白背飞虱产卵量与对照相比明显下降($P<0.05$)。【结论】硅 + 磷喷施处理促进水稻叶片抗虫物质含量增加,硅喷施抑制了白背飞虱的产卵量。

关键词: 白背飞虱; 叶面施用; 硅; 磷; 水稻叶片; 硅含量; 草酸含量; 生殖力

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